

# **AMB Express**

## **Additional File 1**

### **Immunization with recombinant fusion of LTB and linear epitope (40-62) of epsilon toxin elicits protective immune response against the epsilon toxin of *Clostridium perfringens* type D.**

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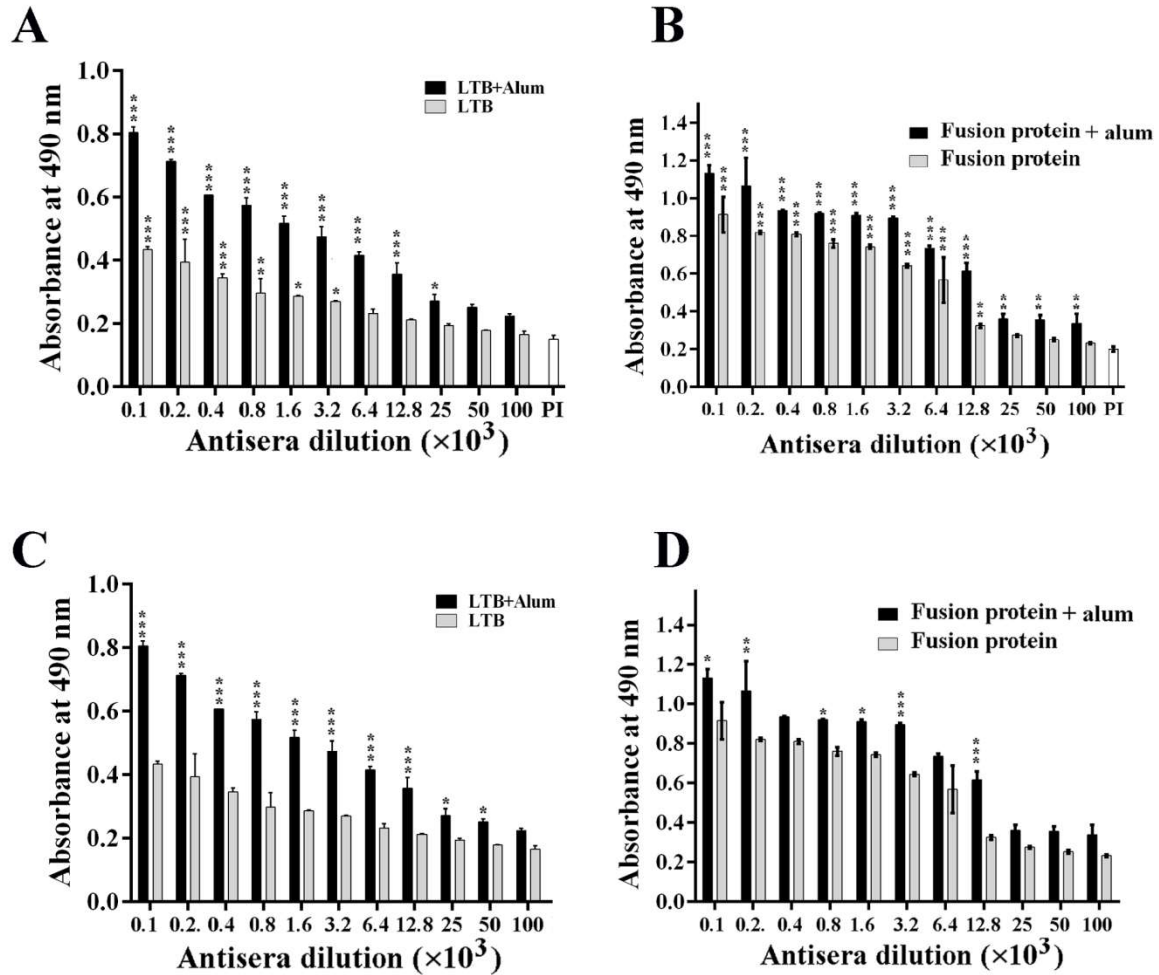
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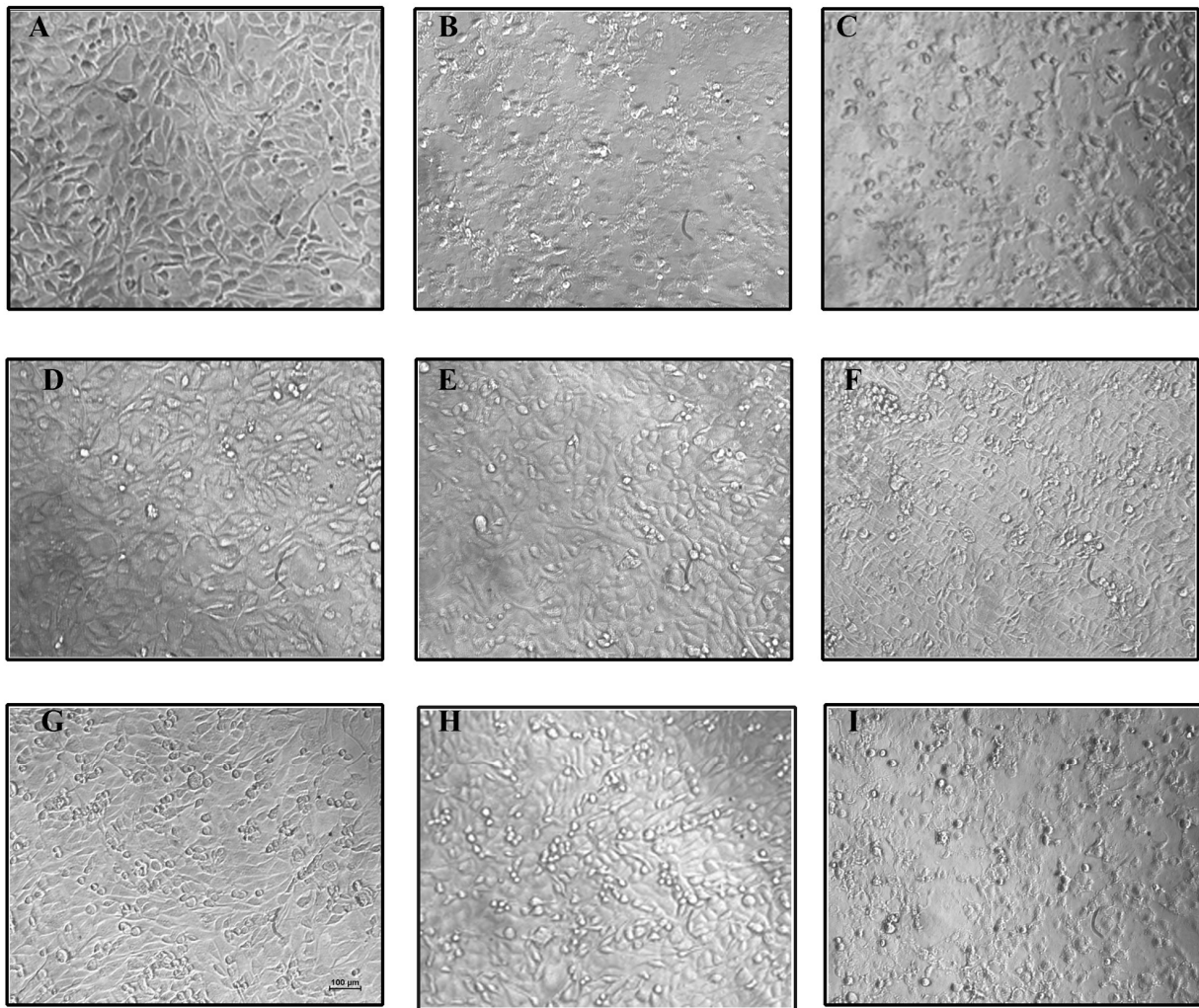
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**Fig. S1. Antibody titer determination:** ELISA to determine antibody titers in the antisera from mice immunized with (A) LTB alone and LTB with alum as adjuvant and (B) Fusion protein rLTB.Etx<sub>40-62</sub> alone and Fusion protein rLTB.Etx<sub>40-62</sub> with alum as adjuvant. As evident from the figure, significant immune response could be generated when the LTB and fusion protein were administered by themselves, signifying the self-adjuvanting activity of the LTB. Immunization of these proteins with alum as adjuvant resulted in an increase in the immune response (Figures S1C and S1D).

Figures S1A and S1B show the statistical difference ( $p$  value) calculated using ordinary two-way ANOVA with respect to pre-immune (PI) serum. Figures S1C and S1D show the statistical difference ( $p$  value) between the antisera generated with alum as compared to that generated without alum. \*,  $p \leq 0.05$ ; \*\*,  $p \leq 0.005$ ; \*\*\*,  $p \leq 0.001$ .



**Fig. S2. Microscopic analysis of MDCK cells treated with the rEtX pre-incubated with anti-rLTB.Etx40-62 antisera:** The rEtX (7.5 ng/5  $\mu$ l) was pre-incubated with equal volume of different dilutions of the anti-rLTB.Etx<sub>40-62</sub> antisera collected after the 2<sup>nd</sup> booster for 1 h at 37°C prior to the addition to MDCK cells. The MDCK cells ( $2 \times 10^4$ /100  $\mu$ l/well) were treated with the rEtX alone or antisera-toxin mixture at for 2 h at 37°C and 5% CO<sub>2</sub> in a humidified incubator. **(A)** Control cells in the culture medium. **(B)** Cells treated with the rEtX. **(C)** Cells treated with rEtX pre-incubated with equal volume of preimmune sera. **(D-I)** Cells treated with the rEtX preincubated with different dilutions of anti-rLTB.Etx<sub>40-62</sub> antisera (D, neat; E, 1:10; F, 1:25; G, 1:50; H, 1:100 and I, 1:500). Images are acquired at 20 $\times$  magnification using a light microscope.